ANTIGEN-BINDING CONSTRUCTS TARGETING HER2

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 15/572,364, filed Nov. 7, 2017 (pending), which is a U.S. National Phase Application of International Application No. PCT/CA2016/050546, filed May 13, 2016, which claims the benefit of U.S. Provisional Application No. 62/161,114, filed on May 13, 2015 and U.S. Provisional Application No. 62/267,247, filed on Dec. 14, 2015, all of which are hereby incorporated in their entirety by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 3, 2021, is named ZWI-030USD1_Sequence_Listing.txt, and is 823,973 bytes in size.

BACKGROUND

[0003] The human epidermal growth factor receptor (HER, erbB) family includes EGFR (HER1), HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4) and the activity of this receptor family regulates the development and maintenance of normal tissue. Overexpression of and/or aberrant regulation of the activity of this receptor family have been implicated in the development and growth of human tumor cells. Members of this family have become targets for the development of therapeutic antibodies for the treatment of cancers. For example, trastuzumab (Herceptin™) and pertuzumab (Perjeta™) are anti-HER2 antibodies that have been developed for the treatment of breast cancers expressing high levels of HER2 (HER2 3+), as measured by the Herceptest™, while T-DM1 (Kadcyla™), a maytansine conjugate of trastuzumab, has also been developed for the treatment of these types of breast cancers.

[0004] Therapeutic antibodies targeting HER2 are disclosed in WO 2012/143523 to GenMab and WO 2009/154651 to Genentech. Antibodies are also described in WO 2009/068625 and WO 2009/068631.

[0005] Mutagenesis of Fab2C4 (pertuzumab) is described in the following publication: Vajdos et al (2002) J. Mol. Biol. 320:415-428. It is also described in US Patent Publication No. US20070117126, to Genentech, published May 24, 2007.

[0006] Methods have been described to increase the affinity of an antigen-binding polypeptide for its antigen. Examples of such methods are described in the following references, Birtalan et al. (2008) *JMB* 377, 1518-1528; Gerstner et al. (2002) *JMB* 321, 851-862; Kelley et al. (1993) *Biochem* 32(27), 6828-6835; Li et al. (2010) *JBC* 285(6), 3865-3871, and Vajdos et al. (2002) *JMB* 320, 415-428.

[0007] Co-owned patent application number PCT/US2014/037401 (WO 2014/182970) describes HER2 antibodies. Co-owned patent application number PCT/CA2013/050358 (WO 2013/166604) describes single arm monovalent antibodies. Co-owned patent applications PCT/CA2011/001238, filed Nov. 4, 2011, PCT/CA2012/050780, filed Nov. 2, 2012, PCT/CA2013/00471, filed May 10, 2013, and PCT/CA2013/050358, filed May 8, 2013 describe therapeutic antibodies. Each is hereby incorporated by reference in their entirety for all purposes.

SUMMARY OF THE INVENTION

[0008] Described herein are antigen-binding constructs comprising a first antigen-binding polypeptide construct which monovalently binds a first HER2 (human epidermal growth factor receptor; a ECD2 (extracellular domain 2) antigen, comprising a heavy chain variable (VH) domain of a 2C4 antibody and/or a light chain variable (VL) domain of a 2C4 antibody, said VH domain and/or said VL domain comprising one or more amino acid modifications as compared to a parent 2C4 antibody sequence whereby the first antigen-binding polypeptide construct has an affinity for the first HER2 ECD2 antigen that is at least 2-fold greater than the affinity of the parent 2C4 antibody for the first HER2 ECD2 antigen; and one or more amino acid modifications selected from one or more framework amino acid modifications at position 74 or position 75 in the VH domain, or at position 49 in the VL domain, where the framework amino acid modification is selected from S74W (H_S74W), S74A (H_S74A), S74F (H_S74F), S74Y (H_S74Y), S74V (H_S74V), S74I (H_S74I), S74L (H_S74L), K75E (H_K75E), K75D (H_K75D), K75V (H_K75V), K75I (H_K75I), K75A (H_K75A), K75L (H_K75L), K75Y (H_K75Y), K75F (H_K75F) and K75W (H_K75W) in the VH domain and Y49W (L_Y49W) or Y49F (L_Y49F) in the VL domain; one or more CDR amino acid modifications selected from T30X in the VH domain CDR1, G56X in the VH domain CDR2, S99X in the VH domain CDR3, and Y96G (L_Y96G) in the VL domain CDR3, wherein X is an amino acid residue having a side chain volume that is greater than that of the wild-type amino acid residue, and combinations therein, wherein the numbering of amino acid residues is according to the Kabat numbering system.

[0009] In some embodiments, T30X is T30Q (H_T30Q), T30N (H_T30N), T30Y (H_T30Y), or T30F (H_T30F); G56X is G56Y (H_56Y) or G56F (H_56F), and S99X is S99W (H_S99W).

[0010] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct has an affinity for the first HER2 ECD2 antigen that is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 100, 150, 200, 250, or 300 -fold greater than the affinity of the parent 2C4 antibody for the first HER2 ECD2 antigen. In some embodiments, the affinity is determined by, e.g., SPR (surface plasmon resonance).

[0011] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises at least 2 amino acid modifications, at least 3 amino acid modifications, or at least 4 amino acid modifications.

[0012] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises one or more amino acid modifications in the framework region, said one or more amino acid modifications selected from S74W (H_S74W), S74A (H_S74A), S74F (H_S74F), S74Y (H_S74Y), S74V (H_S74V), S74I (H_S74I), S74L (H_S74L), K75E (H_K75E), K75D (H_K75D), K75V (H_K75V), K75I (H_K75I), K75A (H_K75A), K75L (H_K75L), K75Y (H_K75Y), K75F (H_K75F) and K75W (H_K75W) in the VH domain and Y49W (L_Y49W) or Y49F (L_Y49F) in the VL domain, and combinations thereof.